

BUDS INDUCED IN CLYMENELLA TORQUATA BY IM-  
PLANTS OF NERVE CORD AND NEIGHBORING  
TISSUES DERIVED FROM THE MID-BODY  
REGION OF WORMS OF THE  
SAME SPECIES

LEONARD P. SAYLES

(From the College of the City of New York and the Marine Biological Laboratory,  
Woods Hole)

In an endeavor to learn more concerning the factors involved in the determination of the type of structure which will develop when a part of the body is lost, a variety of transplantation experiments have been carried out on the polychaete *Clymenella torquata*. It has been shown that when pieces of nerve cord with neighboring tissues from non-regenerating donors are inserted in the body wall of intact hosts, buds are frequently induced. An implant derived from the anterior end (segments 1-7) of a donor may give rise to a bud of either head or tail type (Sayles, 1940a). Whether a head or a tail forms depends to a large extent upon the segment of the host into which the implant is inserted. In the region between segments 1 and 9, head buds have been induced by anterior implants in about one case in six, but no tail buds have ever appeared. When inserted posterior to segment 12, this type of implant does not produce head buds but often results in the formation of an incomplete tail bud with the ventral part of the anal segment missing. The region of segments 10-12 is a transitional zone in which several types of well-differentiated buds may appear, including: (1) heads; (2) tails with the ventral parts of the anal segments missing as at posterior levels; (3) buds each with the ventral half of the anal segment missing and with a cone of new material projecting posteriorly into the open region; (4) buds each with a terminal region consisting of the dorsal half of an anal segment on the open, ventral side of which is a proboscis or similar part.

Implants taken from the posterior region of donors, on the other hand, will not produce head buds, regardless of the level of the host's body at which they are inserted. At posterior levels they produce complete tails. In the transitional zone they give rise to two definite types: (1) complete tails and (2) buds terminating in anal segments of which

only the ventral parts are present. At anterior levels these implants also frequently produce buds terminating in ventral parts of anal segments. In some cases the latter bear extra, conical parts dorsally.

Both anterior and posterior implants exhibit a definite polarity. Definitive head or tail buds are ordinarily not induced by anterior implants when they are inserted with their posterior ends exposed at the surface of the host's body. Posterior pieces, however, must be oriented with their posterior ends out if they are to result in differentiated buds.

The present paper, reporting on results obtained with implants derived from the mid-body region, is intended to complete the description for transplantation experiments in which both donors and hosts are non-regenerating worms. There are reported 277 cases in which im-

TABLE I

*Results of implantation of mid-body pieces with anterior end exposed.*

Level of insertion Source of implant, segments	Anteriorly			In mid-body			Posteriorly			Totals
	8-9	9-10	10-11	8-9	9-10	10-11	8-9	9-10	10-11	
Head buds . . . . .	2	2		4	3	1				12
Double Tail . . . . .			1							1
Tails, open ventrally . . .				2	1		2	3	1	9
Tails, complete . . . . .							5	1		6
Indeterminate buds . . . .	7	4	3	10	6	7			4	41
No new material . . . . .	7	6	7	2	3	2	1	4	6	38
Died within 10 days . . .	2	3		4	3	1	1	4	3	21
Implant lost by host . . .	1	2	1	1				1	1	7
Totals . . . . .	19	17	12	23	16	11	9	13	15	135

plants taken from this mid-body region, segments 8-11, were inserted in the three different regions of the body of *Clymenella*. As in previous experiments, the implants were inserted in the dorso-lateral parts of the segments. This work was carried on at the Marine Biological Laboratory, Woods Hole, during the summers of 1935-1940, inclusive. In the following account most of the buds are described as they were at the time of preservation or discard or, in some cases, a brief time before they were lost.

## RESULTS

The results of 135 cases in which the anterior ends of the implants were exposed are summarized in Table I. The double tail bud consisted of a basal, rounded region bearing two small, partial, anal segments open

ventro-laterally, with the open sides turned slightly toward one another. Each of the buds designated "tail, open ventrally" terminated in a partial anal segment with the ventral half missing. In most of the buds of this type formed at posterior levels the anal-segment regions were large with only a very small mid-ventral region missing in each case. Buds described as "tail, complete" were so called because the anal segments were complete. The indeterminate buds included all of those not possessing at least a clear-cut portion of a head or tail segment. Some of these were elongate with more or less evidence of segmentation but with rounded terminal regions. Others were somewhat conical masses of new material, with occasionally one or more notopodial or neuropodial elements present.

TABLE II

*Results of implantation of mid-body pieces with posterior end exposed.*

Level of insertion	Anteriorly			In mid-body			Posteriorly		Totals
Source of implant. segments	8-9	9-10	10-11	8-9	9-10	10-11	9-10	10-11	
Head buds.....		1	2			2			5
Head-tail buds.....			1			1			2
Tail-cone bud.....			1						1
Tail, open dorsally.....			1		1	5			7
Tail, complete.....						4	3	10	17
Indeterminate buds.....	5	3	7	9	5	16	6	3	54
No new material.....	8	7	3	2	3	7	3	1	34
Died within 10 days.....	2	3	2	3	2	5	2	1	20
Implant lost by host.....	1			1					2
Totals.....	16	14	17	15	11	40	14	15	142

In the remaining 142 operations the posterior ends of the implants were at the surface of the host's body. These are summarized in Table II. Each of the head-tail buds terminated in a weakly differentiated peristomial region and had a partial anal segment, open dorsally, attached in the ventral region of the main bud a short distance from the peristomium. The tail-cone bud was unsegmented and terminated in a large, partial anal segment, the dorsal side of which was missing. On the dorso-lateral side of this anal segment, near its base, there was a small, conical mass of material.

From these data it is clear that implants derived from the level of segments 8-9 possess a distinct polarity. Although 15 definitive head or tail buds resulted from 51 operations in which the anterior ends of these implants were out, none of the 31 cases in which the posterior ends

of the implants were at the surface produced a head or tail bud. It is also interesting that implants from 8-9 produced no tails when inserted at anterior levels, no heads at posterior levels and both types in the transitional region. Both of the mid-body tails were formed at the tenth segment. In all of these respects these implants from segments 8-9 follow rather closely the behavior of implants from anterior sources.

Implants from segments 9-10 of donors, when oriented with their anterior ends out, produced results which resembled those of pieces made up of segments 8-9. The only marked difference was in the distinctly smaller number of tail buds produced by segments 9-10 at posterior levels of hosts. But these implants when inserted with their posterior ends out produced several well-differentiated buds. One was a fairly well formed head which developed at an anterior level, segment 7. One, formed at segment 10, was a small tail bud with only the ventral part of the anal-segment portion present. The other three, produced at segments 13 and 14, were small tail buds with complete anal collars. In distinct contrast with these results are those obtained with anterior pieces inserted with their posterior ends out, an arrangement in which definitive head or tail buds are very rarely produced.

Implants from segments 10-11 showed further transition toward the behavior typical of posterior pieces. Definitive head or tail buds appeared in 3 of 38 cases (about 8 per cent) in which the anterior ends of the implants were exposed. These were of three different types: a double tail, a fairly well-differentiated head, and a bud with only the dorsal part of the anal segment present. On the other hand, 27 out of 72 cases (about 37 per cent) in which the posterior ends of such implants were on the surface gave rise to head or tail buds. At anterior and mid-body levels these belonged to several different types. At posterior levels, however, only tails with complete anal segments were formed. Also the number of differentiated buds produced was much greater in proportion at posterior levels (67 per cent) than in the anterior or transitional regions.

#### DISCUSSION

In general these results with implants from mid-body sources are in accord with what one might expect from a study of buds formed from implants from the anterior and posterior regions of donors. In each case where a tail bud with partial anal segment arose from an implant inserted with its anterior end out, the dorsal part of the anal segment was the portion present regardless of the source of the implant. Evidence has been presented (Sayles, 1940*b*) that the ventral part of the

terminal segment is usually determined by the implant and the dorsal part usually by the host. It is clear, then, that although a partial tail bud may be induced at the anterior end of an implant, the nature of this structure is probably determined by the host. In the case of a partial tail bud produced by an implant with its posterior end out, it was always the dorsal part of the anal segment which was missing. These implants from the mid-body region showed further that the type of bud produced is determined through the interaction of implant and host.

When inserted anterior to the tenth segment, anterior implants produced heads but never tails (Sayles, 1940*a*). Posterior implants inserted in this anterior region produced no heads but frequently tail buds, all of whose anal segments lacked dorsal portions (Sayles, 1940*b*). Mid-body implants made up of the nerve cord of segments 8-9 or 9-10 gave results similar to those following the implanting of anterior pieces here. Pieces of nerve cord made up of segments 10-11, however, showed intermediate behavior. They produced both tails and heads, with the latter being formed even when the posterior ends of implants were exposed. This appearance of heads would not have been predicted from the study of implants from anterior and posterior sources.

In the mid-body region anterior pieces produced both head and tail buds with the latter frequently bearing extra parts which in some cases showed definite head features. Posterior pieces, however, were virtually limited to the formation of tail buds without extra parts. Pieces of nerve cord made up of segments 8-9 or 9-10 gave rise to both head and tail buds, with the latter not bearing extra parts. Again, at this level, implants from segments 10-11 produced both heads and tails.

At posterior levels implants from both anterior and posterior sources produced only tail buds, which in the majority of cases were complete. Similar results were obtained with pieces from the mid-body region.

These results seem to be best explained on the basis of the following theory. There is a strong tail-determining factor in the posterior part of the body. Whenever this is present at full strength, any well-differentiated bud formed will terminate in at least a partial anal segment. This factor is weak or lacking in the anterior part of the body. There is also a head-determining factor, present in the anterior part of the body but either absent in the posterior part of the body or overpowered there by the much stronger tail-determining factor. In the mid-body region (segments 9-11) there is a transition between these conditions found at anterior and posterior levels. This transition may in some way be associated with a general change in the body at the 9-10 intersegmental level. At this point there is a sharp line of demarcation anterior

to which the integument is thicker and richer in glands and the longitudinal muscle bundles are much larger than in the region posterior to this level. No doubt there also exist other, less obvious differences which may be related to the determination of regenerating or induced buds.

That the tail-determining factor at maximum strength is distinctly stronger than the head-determining factor at its maximum is shown by two types of experiments. Implants from the anterior, head-forming region, inserted in the posterior tail-forming part of the host, induce only tail buds, never heads. Implants from the posterior region, when inserted in the anterior part of hosts, also induce only tail buds.

It seems advisable to leave any further discussion of the significance of these results, using implants from the several regions of intact donors, until after presentation of data from experiments already completed using regenerating worms as donors or hosts. Reports on this work with regenerates are now being prepared for publication. An abstract (Sayles, 1941) covering part of the material has already appeared.

#### SUMMARY

The data presented in the present paper and in the two related papers previously published (Sayles, 1940*a* and *b*) may be summarized as follows.

When oriented with their anterior ends out: (1) implants which include the eighth or more anterior segments will produce definitive head or tail buds with the type dependent to a considerable extent upon which segment of the host is involved; (2) implants including only segment 9 or segments 9–10 will also produce both general types of buds but probably not as frequently as implants from more anterior sources; (3) implants from segment 10 or segments 10–11 occasionally form a head or tail bud; (4) posterior implants which do not include any material from a level anterior to segment 11 very rarely, if ever, produce determinate buds.

When oriented with their posterior ends out: (1) anterior implants involving no cord posterior to the ninth segment produce no head or tail buds; (2) implants including only segment 10 or segments 9–10 rarely give rise to these buds; (3) implants including only segment 11 or segments 10–11 give rise to head and tail buds in many cases; (4) posterior implants including only segment 11 or more posterior segments produce tail buds frequently, but head buds rarely, if ever.

These data indicate that: (1) There is a tail-determining factor which is strong in the posterior half of the body but weak or absent at the

level of segments 1-8. (2) There is a head-determining factor which is strongest in the anterior region of the body, weaker in the mid-body region, and perhaps finally absent in the posterior part of the worm. (3) The tail-determining factor at maximum strength is distinctly stronger than any head-determining factor in *Clymenella*.

#### LITERATURE CITED

- SAYLES, L. P., 1940a. Buds induced by implants of anterior nerve cord and neighboring tissues inserted at various levels in *Clymenella torquata*. *Biol. Bull.*, **78**: 298-311.
- SAYLES, L. P., 1940b. Buds induced by implants of posterior nerve cord and neighboring tissues inserted at various levels in *Clymenella torquata*. *Biol. Bull.*, **78**: 375-387.
- SAYLES, L. P., 1941. Implants consisting of young buds, formed in anterior regeneration in *Clymenella*, plus the nerve cord of the adjacent old part (abstract). *Biol. Bull.*, **81**: 302.